Nuclear DNA and Developmental Rate in Frogs

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Goin et al. (1968) described a relation between the time spent as a tadpole in 21 species of frogs and their diploid nuclear DNA amount. The interval from hatching to metamorphosis is from two to four weeks in frogs with low nuclear amounts of DNA (*Scaphiopus, Hyla septentrionalis*) whereas species of *Rana* with high nuclear DNA amounts may overwinter as tadpoles. The tadpole stage is subject to a variety of factors that influence its duration, such as temperature and differences in the stage at hatching.

Early embryonic development can be timed much more accurately and the strong dependence of embryonic developmental rate on temperature allows adjustments for temperature effects (Bachmann, 1969). When the time elapsed at 20°C between the two-cell stage and early tail bud stage (stage 16 of Pollister and Moore, 1937) is compared with nuclear DNA amounts in eleven species of frogs for which such data exist, there results a straight-line relation (Bachmann, in preparation).

Such striking correlation between nuclear DNA and developmental rate could be of considerable significance if it holds true for all frogs. Precise data on developmental rates under controlled conditions are rare and all species for which such data have previously been available are found in relatively mesic regions. Recently, the temperature dependence of developmental rate has been determined for several anuran species of the arid southwestern United States (Zweifel, 1968). Since no species from such an environment was in the earlier sample, they presented particularly suitable material for another test of the relation between nuclear DNA and developmental rate.

Experimental Methods

Specimens of *Bufo cognatus, B. debilis, B. punctatus, Rana pipiens, Scaphiopus bombifrons, S. couchi,* and *S. hammondi* were obtained from the vicinity of the Southwestern Research Station at Portal, Arizona. Nuclei were prepared from kidney and liver tissue fixed in a 3:1 mixture of ethanol and glacial acetic acid for two

hours and from liver tissue fixed in ice cold 10 per cent formalin by homogenizing in a 0.1 per cent solution of Tween 80. Washed free from the detergent the nuclei were spread on slides, air dried, and stored dry until staining. Feulgen staining was preceded by hydrolysis in 5 N HCl at room temperature for prolonged periods of time (30, 50, 60, 90 or 100 minutes) or in 1 N HCl at 60°C for 15 minutes. Total dye bound by individual nuclei was measured at 550 nm on a Barr and Stroud Integrating Microdensitometer. In order to convert the absorption measurements to picograms (pg) of DNA, specimens of *Bufo bufo, B. marinus, B. terrestris,* or *Rana spenocephala* were included in the experiments. Approximate diploid DNA amounts for these species are 14.6, 11.3, 11.1, and 15.0 pg respectively (Bachmann, 1970 a, b).

Developmental rates for the anurans of Portal, Arizona, have been published (Zweifel, 1968). Determinations of the time interval between the two-cell stage and beginning of gill circulation (stage 20 of Pollister and Moore, 1937) have been taken from that publication. Time intervals between the two-cell stage and stage 16 determined by Dr. Zweifel in the same experiments are published here for the first time. These allow comparison with data on other species compiled by Bachmann (1969).

TABLE 1
Diploid nuclear DNA amounts in eleven species of anurans.

Species	Relative Nuclear DNA Amount		pg DNA	Specimens	Determinations
Bufo bufo	8.19 ±0.69	±0.31	15.5	2	5
Bufo cognatus	5.91 ±0.49	±0.25	11.2	2	4
Bufo debilis	5.59 ±0.22	±0.09	10.6	2	6
Bufo marinus	5.77 ±0.07	±0.04	10.9	1	3
Bufo punctatus	5.61 ±0.65	±0.27	10.6	2	6
Bufo terrestris	5.75 ±0.42	±0.14	10.9	5	10
Rana pipiens	9.48 ±0.34	±0.20	17.9	1	3
Rana sphenocephala	7.97 ±0.71	±0.29	15.0	2	6
Scaphiopus bombifrons	1.50 ±0.15	±0.06	2.8	2	6
Scaphiopus couchi	1.95 ±0.07	±0.02	3.6	3	11
Scaphiopus hammondi	1.62 ±0.07	±0.03	3.1	2	6

The columns represent relative DNA amount with standard deviation of the sample and standard error of the mean, absolute DNA amounts in picograms calculated from these, and number of specimens and preparations.

Results and Discussion

Table 1 lists the results of the DNA determinations. Repeated determinations on nuclei from different specimens isolated after either formalin or ethanol-acetic fixation, and stained after a variety of different hydrolysis schedules lead to rather large variation in the results, but tend to cancel out systematic errors introduced by any one preparative method. The DNA values obtained here therefore represent a clear improvement over our earlier estimates (Goin *et al.*, 1968; Bachmann, 1970 a). In particular, the higher ratio between the DNA values for *Bufo bufo* and *Bufo marinus* found in these determinations agrees well with a higher ratio found by Ullerich (1966). The measurements also confirm our earlier finding of the virtual identity of nuclear DNA values in *B. terrestris* and *B. marinus* and reaffirm the value of 15 pg DNA for the diploid DNA amount of *R. sphenocephala* (and Eastern *R. pipiens*). Continued recalibration of the DNA values of certain marker species in our laboratory leads to an increasing reliability of our relative DNA determinations and their calibration in absolute units.

On the basis of these calibrations, *Scaphiopus bombifrons* appears to possess the lowest diploid DNA amount ever recorded for an amphibian. Our earlier determination of the DNA amount in *S. holbrooki* (Goin *et al.*, 1968) corresponds to an absolute value of about 3.2 pg and agrees well with the values recorded here. This emphasizes the striking difference between the earlier value of about 7.1 pg for *S. hammondi* (Goin *et al.*, 1968) and the value of 3.1 pg reported here. The difference is too large to be due to measuring error, and may even be suggestive of polyploidy.

All three *Bufo* species from Portal have intermediate DNA values for the genus. This includes *B. debilis*, while the very similar species *B. retiformis* has a markedly higher DNA amount (Bachmann, 1970 a). The high value for the nuclear DNA of Portal *Rana pipiens* is based on determinations made on a single specimen. Variation in nuclear DNA, both in amount and in kind, among different local populations of "*Rana pipiens*" might result in further evidence on the puzzling problem of evolution in this species group.

The DNA values for *Scaphiopus* (about 3 pg), the three *Bufo* species (about 11 pg) and *Rana pipiens* (18 pg) fall into distinct non-overlapping groups. The same grouping of species is found

when developmental times are compared (Zweifel, 1968, p. 48). Table 2 lists Zweifel's determinations of developmental times between the two-cell stage and stage 16 at various temperatures. Values for the developmental times at 20°C have been interpolated from the data using the equation proposed by Bachmann (1969).

Figure 1 summarizes these data and shows the calculated regression lines between developmental time and nuclear DNA amount. It may be noted that both lines have intercepts. This suggests that there is a minimum timing beyond which the nuclear DNA amount exerts its slowing effect. The close relation between developmental timing and nuclear DNA amount is particularly surprising since the mechanism relating the two must act very indirectly, possibly by way of the nucleic acid metabolism of the growing oocyte.

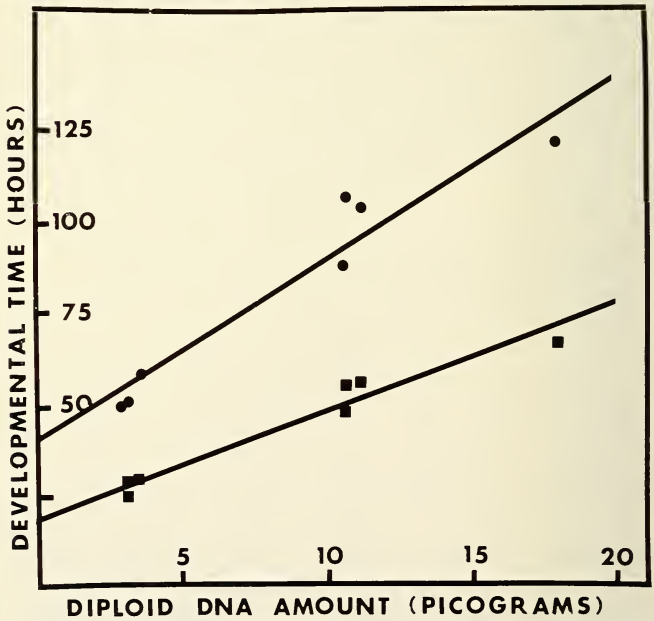

Fig. 1. Developmental rate and nuclear DNA amount for seven anuran species. Times between the two-cell and stage 16 (squares) and stage 20 (dots) against nuclear DNA amounts in picograms.

TABLE 2

Time interval in hours between the two-cell stage and stage 16 in seven species of anurans from Portal, Arizona.

°C	*Bufo cognatus*	*Bufo debilis*	*Bufo punct.*	*Rana pipiens*	*Scaph. bombif.*	*Scaph. couchi*	*Scaph. hammondi*
13.6						91	
15.5					63	63	
15.6	117	101		121			47
	108						
	100						
17.1			77				
18.2		91			33	34	
20.0	(56)	(55)	(48)	(66)	(52)	(29)	(29)
20.3	48					22	
21.0		38					24
21.3							24
24.5	27					15	
	27						
	23						
25.8	25						
26.2			27			11	
31.2	15						
31.5				31			
31.7						8	

These values have been determined by Dr. R. G. Zweifel. Values at 20°C are interpolated from the data.

This is indicated by two observations: (1) polyploid amphibians produced by suppressing the second maturation division do not show an increase in developmental times proportional to the increase in nuclear DNA in every somatic cell; (2) the frog *Ascaphus truei* is the only anuran species known at present which does not fit the DNA-developmental rate correlation. Developmental times are considerably longer (H. A. Brown, cited in Bachmann, 1969) than the relatively low DNA amount of about 7 pg (Macgregor and Kezer, 1970) would suggest. This species is the only anuran species known to have eight functional oocyte nuclei throughout oogenesis (Macgregor and Kezer, 1970). Mediation of the DNA effect on developmental rate by way of oogenesis, for instance through messenger RNA synthesis for early development, would explain the observed correlation as well as these exceptions. The closeness of the correlation in spite of the obviously indirect mechanism involved is

remarkable. A clear relation between nuclear DNA amount and a physiological feature of the whole organism, particularly one of great ecological importance, should subject the nuclear DNA amount directly to natural selection. This may be an indication of one set of factors determining the size of the genome.

Summary

The diploid nuclear DNA amounts of three species of *Scaphiopus,* three species of *Bufo,* and of *Rana pipiens,* all from Portal, Arizona, fall into distinct groups with 3, 11, and 18 pg DNA respectively. The developmental rates of these species from Portal also fall into three distinct groups with *Scaphiopus* showing the fastest rate, *Bufo* developing at intermediate rates, and *Rana* developing slowly. If such diverse factors as environmental adaptation and nuclear DNA amount enter into the determination of the developmental rate of frog species, direct selective effects on genome size can be expected.

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